ated the immune response against viral infection by enhancing the expression of many interferon-inducible genes that were activated by adenovirus carrying β-gal gene compared to the mock treatment.
Conclusion: These data show that Akt controls a genetic program that promotes the activation and survival of endothelial cells. Furthermore, these data provide a framework to understand how Akt signaling controls the blood vessel growth at a molecular level.

1105-73 DNA Microarray Analysis of the Progressive Arterialization and Intimal Hyperplasia of Venous Grafts in a Canine Model

Matthew D. Bacchetta, Ying Zhou, Ronda Chen, Sanjay A. Samy, Arash Salemi, Adam Hamawy, Lufei Hu, Karl H. Krieger, O. Wayne Isom, Wilson Ko, Daniel F. Catanzaro, *New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, New York.*

Intimal hyperplasia of arterialized venous grafts is a leading cause of coronary artery bypass graft (CABG) failure. DNA array analysis of tissue RNA provides a means to identify genes involved in this process. DNA arrays are unavailable for the dog, which is used as a model for CABG surgery. This study evaluated the use of a heterologous DNA array (rat) to measure RNA in vein grafts from dogs. Mongrel dogs underwent bilateral carotid venous interposition grafts and left internal mammary artery (LIMA) graft to a coronary artery. Samples of the grafts were collected for histologic and RNA analyses at 3-, 10-, and 30-days (n=4 per time point). RNA levels were measured using heterologous cDNA microarrays(Affymetrix® rat U34). Each animal served as its own control using a vein sample saved at the initial surgery. Gene expression was analyzed using dChip software. Among the 8,784 genes represented in the probe set, 8.6-10% were assessed as present in the venous mRNA samples. Although lower than the percentage present when RNA was analyzed with GeneChips® of the corresponding species (25-40% for rat vascular tissue in our lab), the levels were highly consistent across samples. Significant changes were found in mRNA levels in arterialized venous grafts for 63 genes (>2-fold, p<0.05). Hierarchical clustering revealed four patterns of gene expression: (1) early stimulation (3 days) followed by suppression (10 & 30 days) (4 genes), (2) early and sustained stimulation (27 genes), (3) early and sustained suppression (36 genes), and (4) mid point stimulation (10 days) flanked by early and late suppression (6 genes). The fold change in mRNA levels ranged from -25 to +20-fold. In addition to known structural (*e. g.* actin, collagen, fibronectin) and regulatory proteins(fos), a number of novel ESTs were also identified. Histological findings (*e. g.* collagen deposition and smooth muscle hyperplasia) were consistent with changes observed in gene expression. This study demonstrates the feasibility of using heterologous (rat) gene chips to analyze the progression of intimal hyperplasia in arterializing canine vein grafts. It also identified possible molecular targets for gene therapy or pharmaceuticals to improve graft life.

1105-74 Profiling Gene Expression in Atherosclerosis Using DNA Microarrays

Kavitha M. Chinnaiyan, Arun Sreekumar, Walter M. Whitehouse, Jr., Naresh T. Gunaratnam, Stuart A. Winston, Arul M. Chinnaiyan, *Saint Joseph Mercy Hospital, Ann Arbor, Michigan, University of Michigan, Ann Arbor, Michigan.*

Background. Atherosclerosis (AS) and its sequelae account for high morbidity and mortality in the U.S. Defining the genes responsible for growth, stability or rupture of the atherosclerotic plaque can aid in understanding its pathogenesis and designing therapies. While traditional approaches focus on one gene at a time, cDNA microarray technology provides a "global" perspective.
Methods. Plaque specimens were collected from routine carotid endarterectomies. Tiny portions of normal adjacent endothelium taken from each patient provided comparison. Control was non-atherosclerotic artery obtained at autopsy. Total RNA extracted from samples was used to generate fluorescently labeled cDNA probes using reverse transcriptase. The probes were hybridized to a high-density, 10K human cDNA microarray (DNA "chip") comprised of > 4,500 known human genes + > 5000 expressed sequence tags (ESTs). Gene expression data deposited into a relational database was analyzed. Hierarchical clustering of data and visualization using the Treeview program defined coordinated patterns of gene expression. Genes differentially expressed were validated by RT-PCR.
Results. >130 genes differentially expressed in plaques relative to normal arterial tissue were identified. Complete concordance was obtained between microarray data and RT-PCR for 18 genes tested. Many genes were previously implicated in AS (e.g., VCAM-1) forming independent validation for our study. Importantly, genes not previously implicated in AS were identified, including OB-Cadherin-11, Cadherin 13, Hevin, Cathepsin O, Kal-1, RBM3 TNR3, LIM7, IGF-2, IGFBP5 and OSF-2. The cluster of 130 genes was classified into distinct functional groups, including cell adhesion and extracellular matrix components, transcription factors, enzymes, complement and MHC, growth factors, kinase and phosphatase, free radical scavengers, glycoproteins and proteoglycans, and miscellaneous.
Conclusion. In this study, we delineated a cohort of novel genes significantly dysregulated in the human atherosclerotic plaque. Future studies are needed to define the functional role of these genes in the pathogenesis of AS and their potential as therapeutic targets.

1105-75 Impairment of Neovascularization by Smoking: Role of HIF-1alpha and VEGF

Sophie Elise Michaud, Catherine Menard, Louis-Georges Guy, Giuseppa Gennaro, Alain Rivard, *University of Montreal, Notre-Dame Hospital, Montreal, Quebec, Canada.*

Background: Smoking is a major risk factor for coronary and peripheral artery diseases. However, the impact of smoking on collateral vessel development has not been studied. Accordingly, we studied the effect of smoking on angiogenesis in the setting of vascular ischemia. **Methods and Results:** Hindlimb ischemia was created by femoral artery resection in mice exposed to cigarette smoke (MES, n=20) and control mice (n=20). We found that smoking was associated with a significant reduction in blood flow recovery as assessed by laser Doppler flow ratio (LDFR) between the ischemic and the normal limb. At day 21 after surgery, MES had a LDFR of 0.60±0.11 vs 0.78±0.07 for controls (p<0.001), and this impaired blood flow perfusion in MES was still present at day 28 after surgery (LDFR: 0.63±0.03 vs 0.80±0.03, p<0.001). CD31 immunostaining confirmed the laser Doppler data by showing a significant reduction in capillary density in the MES at day 28 after surgery (477±34 capillaires/mm^2 vs 681±54 capillaries/mm^2 in controls p<0.05). Western blot analysis of ischemic muscles demonstrated that smoking was associated with a significant reduction in vascular endothelial growth factor (VEGF) expression at days 3, 7 and 14 after surgery. Moreover, this reduced VEGF expression correlated with a significant reduction in the expression (Western blot) and binding activity (electromobility shift assay) of the transcription factor HIF-1α in MES. Lower HIF-1α binding activity and VEGF expression were also observed in vascular smooth muscle cells that were exposed to cigarette smoke extract in vitro. Importantly, rescue of HIF-1α expression using an adenoviral gene delivery strategy resulted in a significant improvement of blood flow recovery in MES. **Conclusion:** 1) Angiogenesis in ischemic vascular disease is impaired by smoking 2) This impairment in neovessel formation is due, at least in part, to the negative effect of smoking on HIF-1α and VEGF expression under hypoxic conditions 3) Smoke-induced impairment of angiogenesis can be rescued by adeno-HIF gene therapy.

1105-76 The Matrix Protein Bone Sialoprotein Enhances Calcification in Vascular Smooth Muscle Cells

Lorraine A. Fitzpatrick, Sean Harrington, Tim O'Brien, *Mayo Clinic, Rochester, Minnesota.*

Background: Matrix proteins are an integral component of the atherosclerotic plaque and regulate calcification. The role of these proteins in the calcification process is poorly understood. Vascular smooth muscle cells (VSMCs) are an integral part of the plaque and produce matrix proteins including osteopontin, osteocalcin, bone sialoprotein and type I collagen. The calcification within an atherosclerotic plaque is an active and regulated process with similarities to ossification in bone. We explored the role of two matrix proteins that are associated with bone calcification, osteopontin (OPN) and bone sialoprotein (BSP). **Methods:** VSMCs were isolated from the coronary arteries of sexually mature pigs. For antisense experiments, oligonucleotides directed at OPN or BSP were made, utilizing the coding strand as a control. A replication defective adenovirus (AD) human serotype 5 was constructed with either OPN (AD-OPN), BSP (AD-BSP), or null (AD-Null) inserts driven by a CMV promoter. VSMCs were infected and maintained in DMEM. VSMCs were fixed and stained for calcium using the Von Kossa method and nodules and calcium were quantified. **Results:** In nontransfected VSMCs, inhibition of BSP expression by oligonucleotides resulted in a decrease in calcified nodules while inhibition of OPN expression caused an increase in calcification. Electron micrographs confirmed the presence of calcium crystals, similar to those found in bone-forming osteoblasts. To further evaluate the role of OPN and BSP, cells were transfected with AD-BSP, AD-OPN or AD-Null. There was a marked and significant increase in nodule calcification in VSMCs containing AD-BSP compared to AD-Null (P<0.02) or AD-OPN (P<0.00001). In contrast, AD-OPN VSMCs revealed marked inhibition of calcified nodules compared to AD-BSP. **Conclusion:** VSMCs and matrix proteins are integral components of the atherosclerotic plaque. These findings suggest that bone sialoprotein and osteopontin contribute to regulation and play an unique role in the calcification process during plaque development.

1105-77 Evaluation of the Dynamics of Substance Delivery via Retrograde Perfusion of the Coronary Sinus in Dogs With Acute and Chronic Cardiac Ischemia

Eduard Fiehn, Wilfried Roethy, Anguo Gu, Kotaro Suehiro, Geng-Hua Yi, Jie Wang, Daniel Burkhoff, *Columbia University - Division of Circulatory Physiology, Department of Medicine, New York City, New York.*

Background: Coronary sinus (CS) retroperfusion (R) is a potential way to deliver substances to the myocardium, particularly in the setting of ischemia where arterial delivery may be limited. We determined optimal conditions to achieve preferential substance delivery to ischemic myocardium with minimal systemic appearance.
Methods: Anesthetized dogs were instrumented for CS-R. Ischemia was induced acutely by LAD ligation (n=14) or chronically by ameroid constrictor (n=3) implanted 3 weeks earlier. The CS was occluded and R performed for 10 min. Colored microspheres (MS) were injected into the CS and their appearance in different regions of the heart and in the kidneys (to index systemic delivery) were quantified. The following parameters were varied: catheter position proximal in CS (n=6) vs distal in the great cardiac vein (n=8); perfusion with blood (n=6) vs crystalloids (n=8); low (5-20 ml/min) vs high (50-250 ml/min) R flow.
Results: During acute ischemia, more MS appeared in ischemic LAD tissue (arterial flow 0.3±0.2 ml/min/g; 4297±2457 MS/g tissue) compared to non-ischemic LCx tissue (arterial flow 1.4±0.3 ml/min/g; 1058±456 MS/g tissue, p<.05). Distal catheter placement in combination with low-flow CS-R reduced the amount of MS shunted to the systemic circulation by 85% compared to proximal placement and high flow perfusion. With chronic LAD occlusion, resting flow was normal in LAD area (1.1±0.3 vs 0.9±0.2 ml/min/g in the LCx area) and CS-R did not provide preferential MS delivery (1133±691 vs 1946±1450 MS/g in the LAD and LCx territories, respectively).
Conclusion: CS-R leads to preferential substance delivery to acutely ischemic areas, but not in the setting of chronic LAD occlusion where resting antegrade blood flow is normal. Systemic appearance of injected substances can be minimized using an optimized retroperfusion protocol. These results will help guide understanding of whether it is advantageous to consider CS-R as a means of delivering a particular substance (e.g., drug, gene, growth factor, etc.) in a particular setting (normal, acute or chronic ischemia).